Cellular Immunology 289 (2014) 150–154

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Increased immunosuppressive treatment combined with unrelated umbilical cord blood infusion in children with severe aplastic anemia

Lin-na Xie, Yuan Fang, Zhe Yu, Ning-xia Song, Fan-sheng Kong, Xi-min Liu, Fang Zhou *

Department of Hematology, The General Hospital of Jinan Military District, Jinan, China

ARTICLE INFO

Article history:
Received 15 February 2014
Accepted 26 March 2014
Available online 3 April 2014

Keywords:
Severe aplastic anemia
Increased immunosuppressive treatment
Umbilical cord blood

ABSTRACT

A combination treatment of unrelated umbilical cord blood (UCB) and increased immunosuppressive treatment (IST) were investigated to reveal the potentially curative therapy for the severe aplastic anemia (SAA). A total of 36 children (2–17 ages) with SAA who received UCB infusion after an IST were analyzed. The treatment consisted of 100 mg/kg cyclophosphamide, 12.5–15 mg/kg antithymocyte globulin and 3 mg/kg cyclosporine. After 3 months, the hematologic complete response (CR) rate was 22.2% and partial response (PR) rate was 38.9%. After 6 months, the CR rate and PR rate was 50.4% and 26.3%, respectively. The probability of 3-year survival was 83.3%. There was no difference in the survival rate either between the horse-ATG and rabbit-ATG or between the SAA and VSAA. The results indicated that the increased IST combined with unrelated UCB infusion has an effective therapeutic potential for children with SAA who lack of compatible donor for transplantation.

1. Introduction

Severe aplastic anemia (SAA) is a life-threatening bone marrow failure disorder characterized by pancytopenia [1]. SAA has been reported to be cured by hematopoietic stem cell transplantation (HSCT) from HLA-matched sibling donor [2], but only a minority of patients have suitable donors. The etiology of aplastic anemia (AA), as well as SAA is still unclear. However, clinical experience and laboratory data of AA indicate that immune-mediated destruction of hematopoietic progenitor cells (HPC) and hematopoietic stem cells (HSC) may be the mechanism of the disease [3]. The immune system suppressing are the alternative therapies for SAA [4,5]. Therefore, immunosuppressive therapy (IST) is generally front-line therapy for most SAA patients.

The IST regimen is usually consisted of anti-thymocyte globulin and cyclosporine A (ATG/CsA) or high-dose cyclophosphamide (CTX) plus CsA. ATG/CsA can induce a hematopoietic response in 60–70% of untreated SAA patients, and the 5 years survival rate is ranges from 60% to 85%. However, almost 40% of patients eventually relapse [6–8]. Meanwhile, treatment of SAA with CTX plus CsA has effectiveness, and is comparable to a conventional regimen and less costly [9]. Furthermore, it may lead to a lower relapse rate or less clonally hematological complications. The higher rates of fungal infection and early mortality deny its application [10]. Many efforts have been done to produce better responses, even though the rare satisfactory results have been obtained up to date [8,11,12]. Although the results mentioned above are disappointing, the increased immunosuppressant is still believed to be the important element for successful IST. The increased immunosuppressant may produce better responses and lower relapse rate, but undeniable, it also produces more toxicity, infection and early mortality. Umbilical cord blood (UCB) is shown to contain sufficient progenitor cells to provide durable engraftment in children, and it provides an alternative stem cell source for patients without matched related or unrelated donors [13,14]. In the present study, an increased IST regimen combined with unrelated UCB infusion was systematically reviewed, which would provide an effective therapy for severe aplastic anemia patients.

2. Materials and methods

2.1. Sample

From January 2005 through January 2011, a total of 36 children (median age 8.5, 19 males and 17 females) with acquired SAA who received the therapy of increased IST regimen plus unrelated UCB infusion at our department were enrolled in this study. Patients with a history of myelodysplastic syndrome (MDS), paroxysmal nocturnal hemoglobinuria (PNH) and congenital AA were

* Corresponding author. Address: Department of Hematology, the General Hospital of Jinan Military, No.25 Shifan Road, 250031 Jinan, Shandong Province, China. Fax: +86 0531 51665316.
E-mail address: fangzhou223@hotmail.com (F. Zhou).

http://dx.doi.org/10.1016/j.cellimm.2014.03.014
0008-8749/

excluded. Meanwhile, a historical control group (from January 2003 to January 2005) including 20 children (median age 7.6, 12 males and 8 females) with acquired SAA received the standard IST regimen without UCB infusion were included. All patients and their guardians provided their written informed consent in accordance with the Declaration of Helsinki, and the study was approved by the institutional Review Board of General Hospital of Jinan Military.

2.2. Definitions

The diagnosis of SAA required bone marrow cellularity less than 30%, and 2 of 3 peripheral blood counts criteria including platelet counts $<20 \times 10^9$/L, neutrophil count $<0.5 \times 10^9$/L, and reticulocyte count $<20 \times 10^9$/L [15]. The diagnosis of VSAA required a neutrophil count $<0.2 \times 10^9$/L [16]. Patients with SAA and VSAA were classified as SAA group and VSAA group, respectively. Complete response (CR) was defined as transfusion independence associated with a hemoglobin level of $\geqslant 110$ g/L, neutrophil count of $\geqslant 1.5 \times 10^9$/L, and a platelet count of $\geqslant 150 \times 10^9$/L. Partial response (PR) was defined as no longer meeting the criteria for SAA and no transfusion dependence for platelets or red blood cells. Continuous transfusion dependency was classified as no response. Relapse was defined as the blood counts decrease to the values that either requiring transfusions or needing reinstitution of immunosuppressive therapy (or HSCT) [7,17].

2.3. Treatment protocol

UCB units were obtained from the Beijing Cord Blood Bank. HLA-A, HLA-B and HLA-DR antigen were selected as the serologically type of UCB units. ABO incompatibility was as minor factor used in UCB selection.

To maintain trough levels (150–250 ng/mL), all patients received conditioning as follows: (i) CTX 100 mg/kg (divided in 2 daily doses) from day −3 to day −2, and (ii) rabbit ATG(r-ATG) 2.5–3 mg/kg/day (or horse ATG, h-ATG 30 mg/kg/day) from days −5 to −1, CsA 3 mg/kg/day intravenously (transitioned to oral) beginning day −1. One UCB unit was transfused on day 0. Since the h-ATG was withdrawn from the local market in 2007 and r-ATG was adopted as upfront treatment, r-ATG (r-ATG group) and h-ATG (h-ATG group) were both involved in the present study. The patients enrolled in the department from 2005 to 2006 were received h-ATG as IST treatment. The control group received rabbit ATG (r-ATG) 2.5–3 mg/kg/day (or horse ATG, h-ATG 30 mg/kg/day) from days −5 to −1, and CsA 3 mg/kg/day intravenously (transitioned to oral) beginning day −1.

Methylpredinisolone (1–2 mg/kg) was given prior to the first dose of ATG for 5 days, and then the dose was tapered over in the subsequent 5 days. If the neutrophil count $<0.5 \times 10^9$/L, the granulocyte colony-stimulating factor (G-CSF, 5 μg/kg) was used in addition. When neutrophil count $>1.5 \times 10^9$/L, the dose of G-CSF was slowly tapered. The prophylaxis of graft-versus-host disease (GVHD) consists of the following two parts: (i) methotrexate (MTX) short course (15 mg/m^2) intravenously on day 1 followed by 10 mg/m^2 intravenously on days 3, 6, and 11; (ii) mycophenoate mofetil (MMF) 250 mg/m^2 from day 1 to day 28. Assessment of engraftment, chimerism, GVHD and supportive care during therapy were performed as previously reported [18]. Chimerism status was evaluated with quantitative polymerase chain reaction (q-PCR) analysis for microsatellite DNA markers. Response and chimerism status were assessed at scheduled visits (1–3, 6, 12 months and yearly).

The median follow-up was 68 months (range, 33–103 months). Statistical analyses were performed using SPSS 13.0 (SPSS Inc, Chicago, Ill). Overall survival rate was calculated using the Kaplan–Meier method. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Patients and characteristics

A total of 36 patients were diagnosed as acquired SAA which received modified IST during 7-year period. A total of 7 patients ($n = 7$) received horse ATG as IST treatment from 2005 to 2006. From 2005 to 2007, 12 patients were given GVHD prophylaxis after cord blood transfusion. No patients were found donor engraftment after the assessment of engraftment and chimerism, the remaining 24 patients received CsA only. GVHD prophylaxes such as MTX and MMF were discarded, and no GVHD symptoms were found. Chimerism analysis showed no signs of donor engraftment. Characteristics of the patients and UCB were shown in Tables 1 and 2, respectively.

3.2. Clinical efficacy

The median time of neutrophil (count >0.5 × 109/L), last platelet transfusion, and last red cell transfusion were 25 days (range, 17–61 days), 50 days (range, 12–101 days), and 54 days (range, 16–120 days), respectively. All these results were better than IST group (Table 3). At 3 months, totally 8 patients achieved CR (22.2%) and 14 patients achieved PR (38.9%) in IST + UCB group. The total response rates in months 6, 9 and 12 after treatment were

Table 1
The characteristics of patients and control.

Variables	Patients ($N = 36$)	Control ($N = 20$)	p
Gender (Boy/Girl)	19/17	12/8	0.31
Median age at diagnosis, *y* (range)	8.5 (2–17)	7.6 (3–15)	0.45
Etiology			
Idiopatic	33	19	
Drug	1	0	
Hepatitis	1	1	
Chemical	1	0	
Pretreatment (naïve/CSA + stanozolol)	19/16	13/7	
Hemoglobin, g/dl (range)	58.11 (29–103)	49.23 (25–120)	0.61
ANC, $\times 10^9$ cells/L (range)	0.28 (0–1.08)	0.41 (0–1.43)	0.15
ALC, $\times 10^9$cells/L (range)	1.61 (0.35–3.20)	1.16 (0.51–2.77)	0.23
Platelets, $\times 10^9$cells/L (range)	24.17 (1–119)	42.81 (6–107)	0.08
Reticulocyte, $\times 10^{12}$cells/L (range)	0.014 (0.0011–0.0298)	0.023 (0–0.0421)	0.61
$CD3^+CD4^+/CD3^+CD8^+$	1.17 (0.50–2.43)	0.98 (0.42–3.10)	0.07
Interval from diagnosis to intense-IST (days)	307.08 (22–2050)	401.21 (15–2110)	0.72
SAA/VSAA	17/19	6/14	